

Figure 2

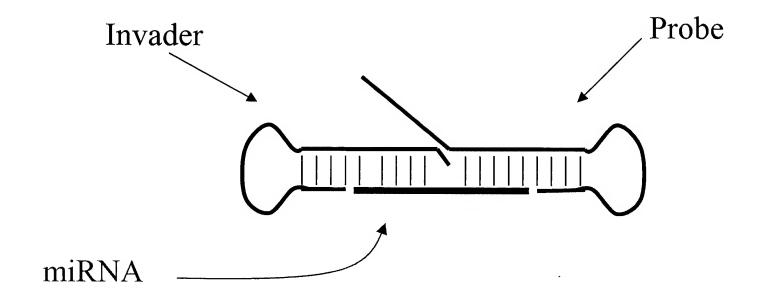
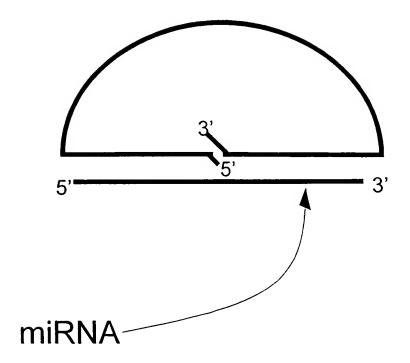
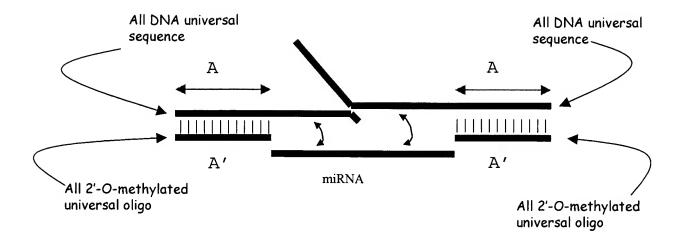


Figure 3



#### Figure 4



A = Universal sequence that is added to the 3' and 5' of probes and INVADER oligonucleotides, respectively.

From 5' to 3', the probe is composed of the 5'-flap, the miRNA complementary region, and the DNA universal sequence "A".

The INVADER oligonucleotide from 5' to 3', is composed of the DNA universal sequence "A" and an miRNA complementary region.

A' = 2'-O-methyl universal oligonucleotide that compliments the sequence "A" and is added to kits as a standard oligonucleotide.

SEQ ID NO	Target	Oligo type	Sequence (5'-3')
SEQ ID NO:1	human let-7 miRNA	Invader oligo	ggcacuuuugugccAACTATACAACCG
SEQ ID NO:2	human let-7 miRNA	probe oligo	CCGTCGCTGCGTTACTACCTCAcgacguuuucgucg
SEQ ID NO:3	human let-7 miRNA	arrestor oligo	cgacgaaaacgucgugagguaguaacgcag
SEQ ID NO:4	human let-7 miRNA	miRNA	ndaaddnaddandnanadnn
SEQ ID NO:5	human let-7 miRNA	Invader oligo	ggcacuuuugugccAACTATACAACT
SEQ ID NO:6	human let-7 miRNA	probe oligo	CCGTCGCTGCGTCTACCTCAcgacguuuucgucg
SEQ ID NO:7	human let-7 miRNA	arrestor oligo	cgacgaaaacgucgugagguaguagacgcag
SEQ ID NO:8	human let-7 miRNA	Invader oligo	ggcacuuuugugccAACTATACAAT
SEQ ID NO:9	human let-7 miRNA	probe oligo	AACGAGGCGCACCTACTACCTCAcgacguuuucgucg
SEQ ID NO:10	human let-7 miRNA	arrestor oligo	cgacgaaaacgucgugagguaggaaggaggugcgc
SEQ ID NO:11	human miR-1	Invader oligo	ggcagcuuuugcugccCTCCATACTTCTC
SEQ ID NO:12	human miR-1	probe oligo	AACGAGGCGCACTTACATTCCAcgagccuuuuggcucg
SEQ ID NO:13	human miR-1	arrestor oligo	cgagccaaaaggcucguggaauguaagugcgc
SEQ ID NO:14	human miR-1	miRNA	uggaauguaagaaguauggag
SEQ ID NO:15	human miR-1	Invader oligo	ggcagcuuuugcugccCTCCATACTTCC
SEQ ID NO:16	human miR-1	probe oligo	AACGAGCGCACTTTACATTCCAcgagccuuuuggcucg
SEQ ID NO:17	human miR-1	arrestor oligo	cgagccaaaaggcucguggaauguaaagugcgc
SEQ ID NO:18	human miR-1	Invader oligo	ggcagcuuuugcugccCTCCATACTTT
SEQ ID NO:19	human miR-1	probe oligo	AACGAGGCGCACCTTTACATTCCAcgagccuuuuggcucg
SEQ ID NO:20	human miR-1	arrestor oligo	cgagccaaaaggcucguggaauguaaaggugcgc
SEQ ID NO:21	FAM FRET	FRET probe	Yca-cXt-gct-tcg-tgg
SEQ ID NO:22	SRT	Secondary Reaction template	CCA GGA AGC AAG TGA CGC AGC GAC ggu
SEQ ID NO:23	human let-7 miRNA	Invader oligo	ggcacuuuugugccaaCTATACAAT
SEQ ID NO:24	human let-7c miRNA	miRNA	nogangangangangangun
SEQ ID NO:25	human let-7e miRNA	miRNA	nddnacdnnddanddadn
SEQ ID NO:26	human let-7f miRNA	miRNA	nndanandnnadandaadn
SEQ ID NO:27	human miR-135	Invader oligo	ccgagcgaaagcucggTTCACATAGGAATC
SEQ ID NO:28	human miR-135	probe oligo	AACGAGGCGCACAAAAAGCCATAcgagccgaaaggcucg
SEQ ID NO:29	human miR-135	arrestor oligo	cgagccunucggcucguanggcunuuugugcgc
SEQ ID NO:30	human miR-135	Invader oligo	ccgagcgaaagcucggTTCACATAGGAAC
SEQ ID NO:31	human miR-135	probe oligo	AACGAGGCGCACTAAAAGCCATAcgagccgaaaggcucg
SEQ ID NO:32	human miR-135	arrestor oligo	cgagccuuucggcucguanggcuuunuagugcgc
SEQ ID NO:33	human miR-135	Invader oligo	ccgagcgaaagcucggTTCACATAGGAC
SEQ ID NO:34	human miR-135	probe oligo	AACGAGGCGCACATAAAAAGCCATAcgagccgaaaggcucg
SEQ ID NO:35	human miR-135	arrestor oligo	cgagccunucggcucguauggcununuaugugcgc
SEQ ID NO:36	human miR-135	Invader oligo	ccgagcgaaagcucggTTCACATAGGC
SEQ ID NO:37	human miR-135	probe oligo	AACGAGGCGCACAATAAAAAGCCATAcgagccgaaaggcucg
SEQ ID NO:38	human miR-135	arrestor oligo	cgagccuuucggcucguauggcuuuuuuuuugggc

FIG 5

SEQ ID NO	Target	Oligo type	Sequence (5'-3')
SEQ ID NO:39	human miR-16	miRNA	uagcagcacgtaaauauuggcg
SEQ ID NO:40	SRT	Secondary Reaction template	CCAGGAAGCAAGTGGAGGGGCGTGACGgu
SEQ ID NO:41	human GAPDH	Invader oligo	ggaaucauauuGGAACATGTAAACCATC
SEQ ID NO:42	human GAPDH	probe oligo	CCGCCGAGATCACGTAGGTTGAGGTC-NH2
SEQ ID NO:43	human GAPDH	arrestor oligo	gaccncaacuacguganc
SEQ ID NO:44	human miR-125b	miRNA	ncccngagacccuaacuuguga
SEQ ID NO:45	U6 RNA	Invader oligo	GGCCATGCTAATCTTCA
SEQ ID NO:46	U6 RNA	probe oligo	CCGCCGAGATCACTCTGTATCGTTC-NH2
SEQ ID NO:47	U6 RNA	arrestor oligo	gaacganacagaguganc
SEQ ID NO:48	RED FRET		Yct-cXt-tct-cag-tgc-g
SEQ ID NO:49	SRT	Secondary Reaction template	CCAGCAAGCAAGTGGTGATCTCGGCggu
SEQ ID NO:50	human let-7a miRNA	probe oligo	CCGTCGCTGCGTCTACTACCTCA-NH2
SEQ ID NO:51	human let-7a miRNA	Invader oligo	AACTATACAACT
SEQ ID NO:52	human let-7a miRNA	probe oligo	CCGTCGCTGCGTTACTACCTCA-NH2
SEQ ID NO:53	human let-7a miRNA	Invader oligo	AACTATACAACCG
SEQ ID NO:54	human let-7a miRNA	arrestor oligo	ngagguaguagacgcag
SEQ ID NO:55	human miR-15	probe oligo	AACGAGGCGCACATGTGCTGCTAcgagccuuuuggcucg
SEQ ID NO:56	human miR-15	Invader oligo	ggcagcuuuugcugccCACAAACCATTC
SEQ ID NO:57	human miR-15	arrestor oligo	cgagccaaaaggcucguagcagcacaugugcgc
SEQ ID NO:58	human miR-15	probe oligo	AACGAGGCGCACATGTGCTGCTAGCTCGCCACGCCG-NH2
SEQ ID NO:59	human miR-15	Invader oligo	GCTCGCCACGCCGCACAAACCATTC
SEQ ID NO:60	human miR-15	stacker oligo	cdacandacaac
SEQ ID NO:61	human miR-15	arrestor oligo	cggcguggcgagcuagcacaugugcgc
SEQ ID NO:62	human miR-15	miRNA	bnbnnnbbneenesesbesben
SEQ ID NO:63	human miR-135	probe oligo	AACGAGGCGCACAATAAAAAGCCATAGCTCGCCACGCCG-NH2
SEQ ID NO:64	human miR-135	Invader oligo	GCTCGCCACGCCGTTCACATAGGC
SEO ID NO:65	human miR-135	arrestor oligo	cggcguggcgagcuauggcuununaugggcgc
SEQ ID NO:66	human miR-15	arrestor oligo	nagcagcacaugugcgc
SEQ ID NO:67	human miR-15	probe oligo	AACGAGGCGCACATGTGCTGCTAGGCGAAGCC
SEQ ID NO:68	human miR-15	Invader oligo	GGCGAAGCCACATTC
SEQ ID NO:69	human miR-15	probe oligo	AACGAGGCGCACATGTGCTAGGCGAAgcc
SEQ ID NO:70	human miR-15	Invader oligo	ggcGAAGCCCACAAACCATTC
SEQ ID NO:71	human miR-15	probe oligo	AACGAGGCGCACATGTGCTGCTAggcuucggcc
SEQ ID NO:72	human miR-15	Invader oligo	ggcuucggccCACAAACCATTC
SEQ ID NO:73	human let-7a miRNA	Invader oligo	GGCACTTTTGTGCCAACTATACAACT
SEQ ID NO:74	human let-7a miRNA	probe oligo	CCGTCGCTGCGTCTACCTCACGACGTTTTCGTCG
SEQ ID NO:75	human let-7a miRNA	Invader oligo	ggcacTTTGTGCCAACTACAACT
SEQ ID NO:76	human let-7a miRNA	probe oligo	CCGTCGCTGCGTCTACTACCTCACGACGTTTTcgucg

SEG ID NO	larget	Oligo type	Sequence (5'-3')
SEQ ID NO:77	human miR-16 miRNA	Invader oligo	ggcagcuuuugcugccCGCCAATATTG
SEQ ID NO:78	human miR-16 miRNA	probe oligo	AACGAGGGCACTACGTGCTAcgagccuuuuggcucg
SEQ ID NO:79	human miR-16 miRNA	arrestor oligo	cgagccaaaaaggcucguagcagcacguagugcgc
SEQ ID NO:80	human miR-125b miRNA	Invader oligo	ggcagcuuuugctgccTCACAGTTAGA
:81	human miR-125b miRNA	probe oligo	AACGAGGCGCACGGTCTCAGGGAcgagccuuuuggcucg
SEQ ID NO:82	human miR-125b miRNA	arrestor oligo	cgagccaaaaggcucgucccugagaccgugcgc
SEQ ID NO:83	human let-7a miRNA	probe oligo	CCGTCGCTCTACTACCTCAcgacguuuucgucgu
SEQ ID NO:84	human let-7a miRNA	Invader oligo	uggcacuuuugugccAACTATACAACT
SEQ ID NO:85	human let-7a miRNA	probe oligo	CCGTCGCTGCGTCTACTACCTCAcgacguuuucguc
SEQ ID NO:86	human let-7a miRNA	Invader oligo	gcacuuuugugccAACTATACAACT
			nenoeeönööocoocoocoocoocoocoocoocoocoocoocoocooc
SEQ ID NO:87	precursor human let-7a	miRNA	dcaannnncnaccnnnccndaadnccc
SEQ ID NO:88	miR-124a 21nt	miRNA	uaaggcacgcggugaaugcca
SEQ ID NO:89	miR-124a 22nt	miRNA	nnaaggcacgcggugaangcca
SEQ ID NO:90	miR-124a miRNA	probe oligo	CCGTCGCTGCGTGCCTTAcgagccuuuuggcucg
SEQ ID NO:91	miR-124a miRNA	arrestor oligo	naaggcacgcgacgcag
SEQ ID NO:92	miR-124a miRNA	Invader oligo	ggcagcuuuugcugccTGGCATTCACA
SEQ ID NO:93	U6 RNA	probe oligo	CCGCCGAGATCACCTTCTCTGTAT-NH2
SEQ ID NO:94	U6 RNA	Invader oligo	CATCCTTGCGCAGGGCCATGA
SEQ ID NO:95	U6 RNA	arrestor oligo	auacagagaauuaggugauc
SEQ ID NO:96	human miR-135	miRNA	nanddennnnannecnandndaa
0:97	human miR-1d	miRNA	nddaandnaaadaadnandnan
SEQ ID NO:98	human miR-1d	probe oligo	AACGAGGCGCACTTTACATTCCAcgagccuuuuggcucg
SEQ ID NO:99	human miR-1d	Invader oligo	ggcagcuuuugcugccATACATACTTCC
SEQ ID NO:100	human miR-1d	arrestor oligo	cgagccaaaaggcucguggaauguaaagugcgc
SEQ ID NO:101	human beta actin siRNA	probe oligo-antisense	AACGAGGCGCACAAGATCATTGCggcuucggcc
SEQ ID NO:102	human beta actin siRNA	Invader oligo-antisense	ggcuucggccAATGAAGATCC
SEQ ID NO:103	human beta actin siRNA	arrestor oligo-antisense	gcaangancungugcgc
SEQ ID NO:104	human beta actin siRNA	probe oligo-sense	AACGAGGCGCACCTTGATCTTCAggcuucggcc
SEQ ID NO:105	human beta actin siRNA	Invader oligo-sense	ggcuucggccAAGCAATGATA
SEQ ID NO:106	human beta actin siRNA	arrestor oligo-sense	וומששמשוומנמנ

Figure 6
Design Optimization

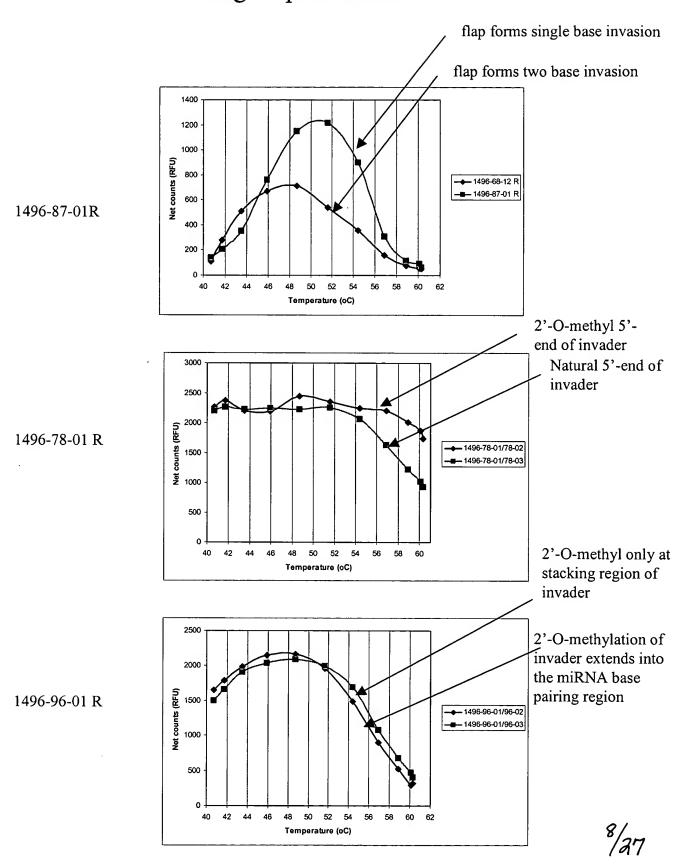


Figure 7
Design Optimization

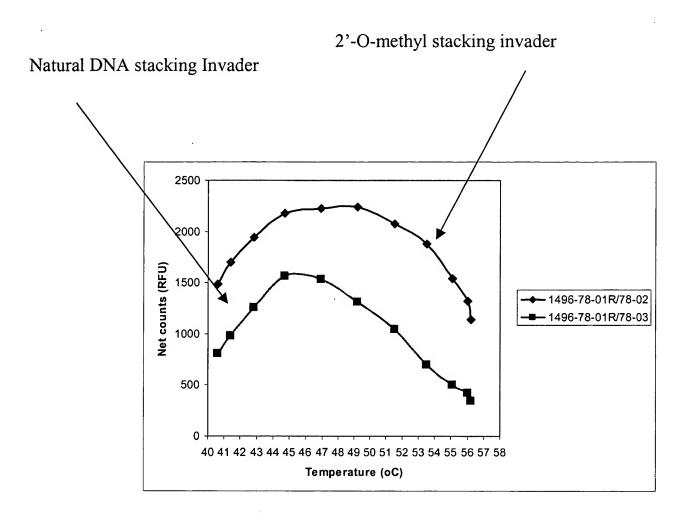
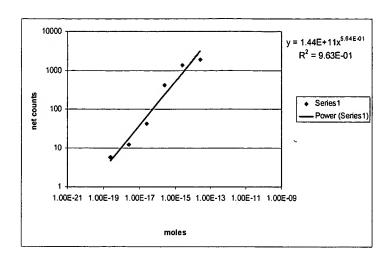
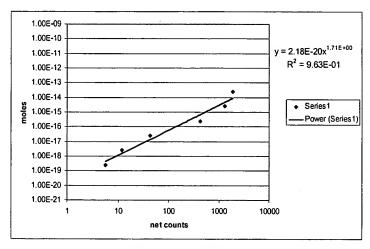


Figure 8 LOD let-7 (1496-78-01R)





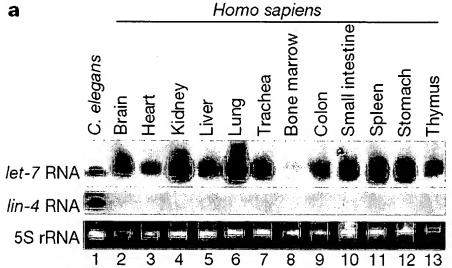
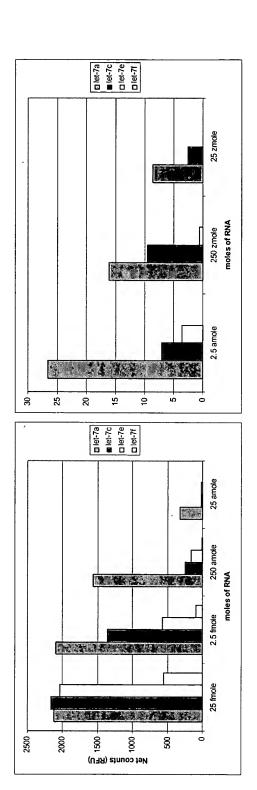


Figure 9 cross reactivity let-7



### Figure 10 LOD mir-1

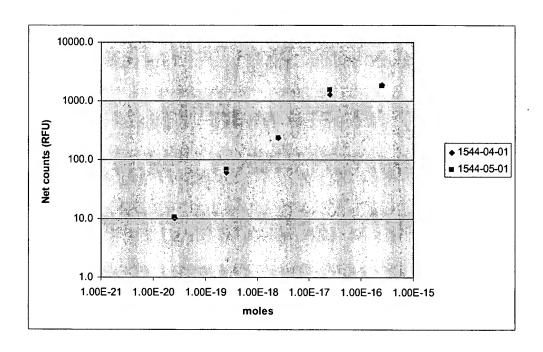
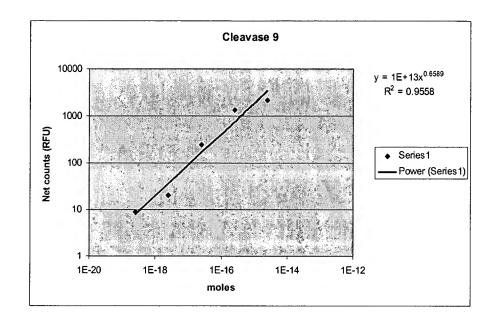
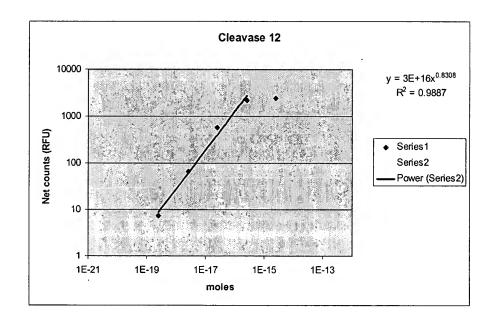


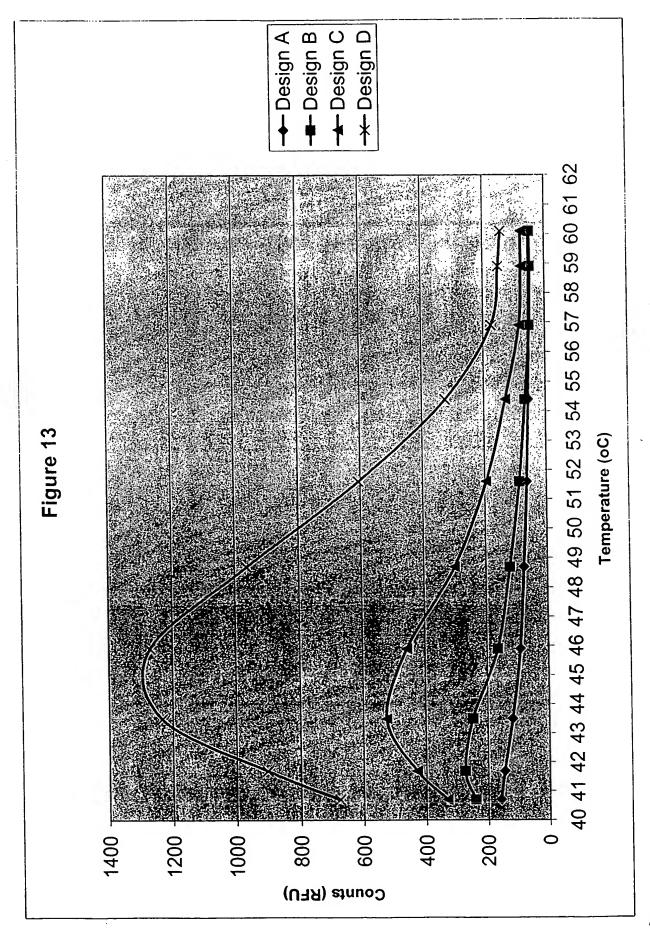
Figure 11 LOD let-7 (1496-78-01R) using CLEAVASE XII enzyme





## FIGURE 12

110								
H —	TTTT	TTTT.	TTTT.	TLT.	TTTT.	TLL-	TTTT.	'TTT-
0	CAAAT	CAAAT	CATAT	CATAT	CATAT	CATAT	CACAT	CAAAT
100	CGTTC	SCGTTC	SCGTTC	SCGTTC	SCGTTC	CGTTC	CGLIC	ATGGTC
06 —	STGAAC	STGAAC	STGAAC	STGAAC	STGAAG	STGAAC	STGAAG	SAGAAZ
	AATTC	AATTC	AATTC	AATTC	AATTC	AATTC	AAATC	AAATC
o	GITCTTCCG-AGAACATATACTAAAATTGGAACAATACAGAGAATTAGCATGGCCCCTGCGCAAGGATGACACGCA-AATTCGTGAAGCGTTCCAAATTTTT	GTTCTTCCG-AGAACATATACTAAAATTGGAACAATACAGAGAAGATTAGCATGGCCCCTGCGCGAAGGATGACACGCA-AATTCGTGAAGCGTTCCAAATTTTT	<b>GTGCTCGCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCA-AATTCGTGAAGCGTTCCATATTTT</b>	GTGCTCGCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCA-AATTCGTGAAGCGTTCCATATTT-	GTGCTTGCTTCGGCAGCACATATACTAAAATTTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCA-AATTCGTGAAGCGTTCCATATTTTT	GFGCCTGCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCA-AATTCGTGAAGCGTTCCATATTT-	IGTTCTTGCTTCGGCAGAACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCAGCGCAAGGATGACACGCA-AAATCGTGAAGCGTTCCACATTTTT	GTCCCTTCGGGGACATCCGATAAAATTGGAACGATACAGAGAAAGATTAGCATGGCCCCTGCGCAAGGATGACGACGCATAAATCGAGAAATGGTCCAAATTTT-
~	ATGAC!	ATGAC!	ATGAC!	ATGAC!	ATGAC!	ATGAC!	ATGAC!	ATGACI
70	SCAAGG	SCAAGG	CAAGG	CAAGG	CAAGG	CAAGG	SCAAGG	SCAAGG
	CCTGCG	CCTGC	CTGC	CCTGCC	CTGC	CTGC	CCAGCG	CCTGCC
9	TGGCC	TGGCC	TGGCC	TGGCC	TGGCC	TGGCC	TGGCC	TGGCC
	TTAGCA	<b>LTAGCA</b>	<b>LTAGCA</b>	<b>LTAGCA</b>	LTAGCA	<b>LTAGCA</b>	FTAGCA	FTAGCA
50	GAAGAT	GAAGA.	GAAGAT	GAAGAT	GAAGAT	GAAGAT	GAAGAT	GAAGAT
	ACAGA	'ACAGA	'ACAGA	'ACAGA	'ACAGA	ACAGA	ACAGA	ACAGA
4	AACAAT	AACAAT	AACGAT	ACGAT	ACGAT	ACGAT	ACGAT	AACGAT
0	ATTGG	ATTGG	ATTGG2	ATTGG2	ATTGG7	ATTGG	ATTGG2	ATTGG2
30	CTAAA	CTAAA	CTAAA	CTAAA	CTAAA	CTAAA	CTAAA	ATAAA
0	CATATA	CATATA	CATATA	CATATA	CATATA	CATATA	SATATA	PATCCG
.,	-AGAA	-AGAA(	CAGCAC	CAGCAC	CAGCAC	CAGCAC	CAGAAC	GGA(
10	TTCCG	TICCG	TTCGG	TTCGG	TTCGG	TTCGG	TTCGG	TTCGG
	GTTC	GTIC	SCTCGC	SCTCGC	CTTGC	CCTGC	ICTIGO	-GICCC
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		a)					æ	is
	C.elegans	C.briggsae	ម្ព	ě	Xenopus		Drosophila	Arabidopsis
	C.e.	C.b	human	monse	Xenc	Rat	Dros	Arak



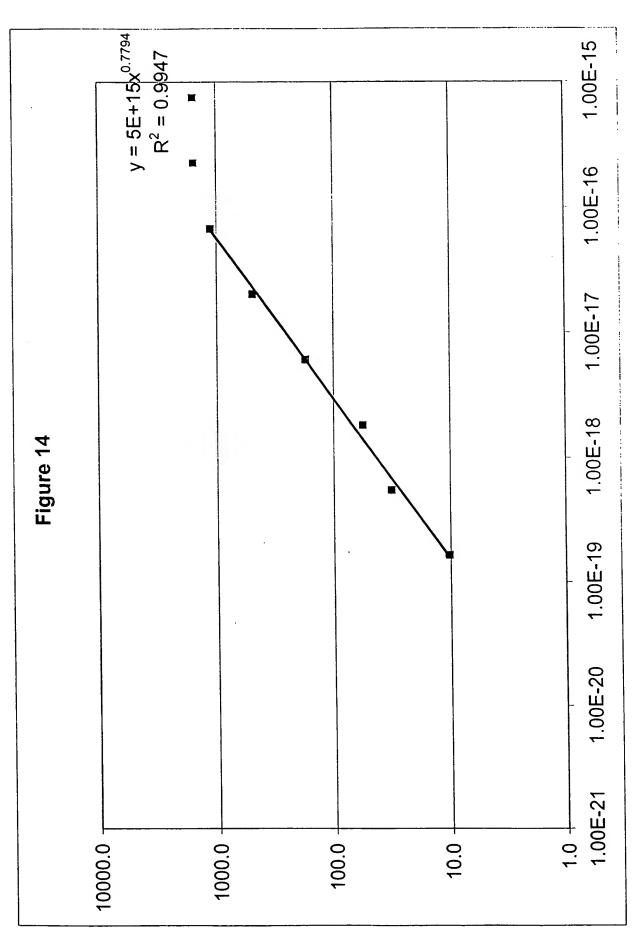


Fig. 15

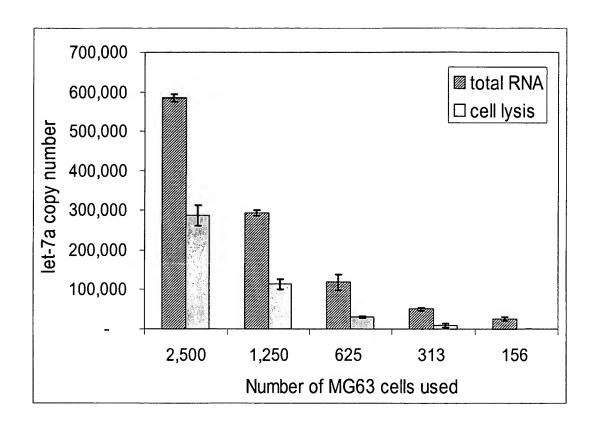


Figure 16

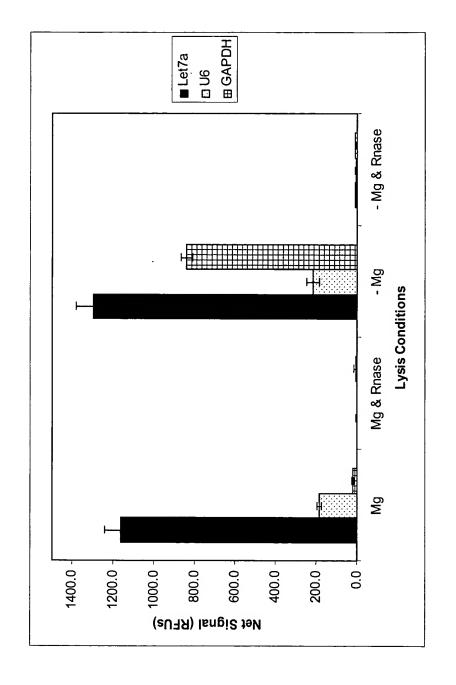
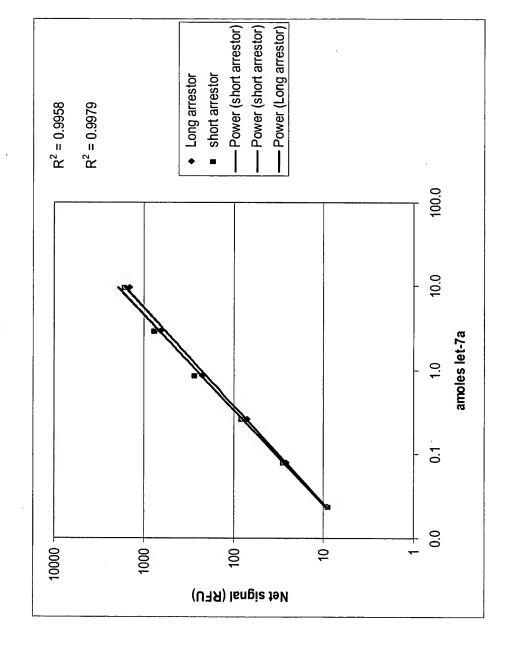
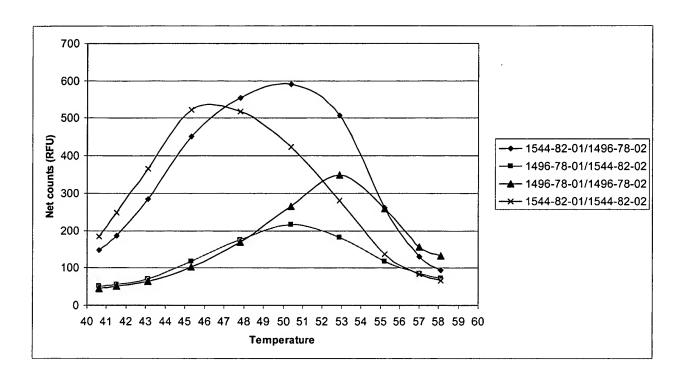


Figure 17



#### FIGURE 18



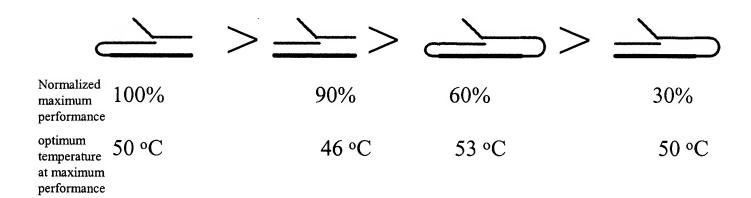


Fig. 19

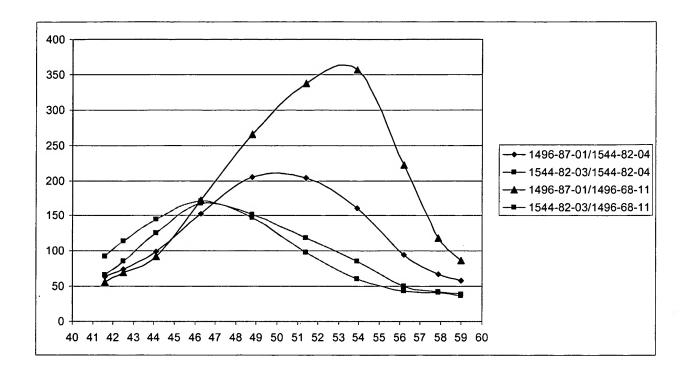
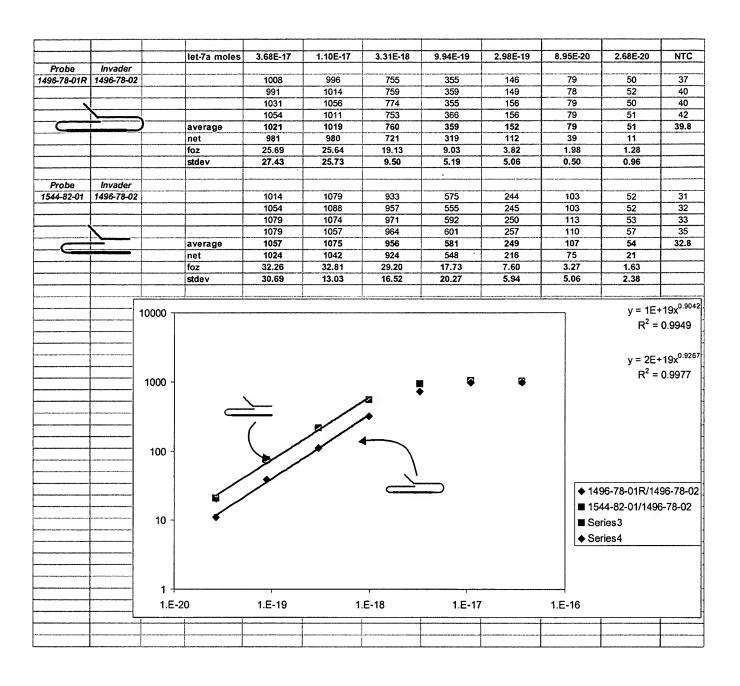


Fig. 20



## Figure 21

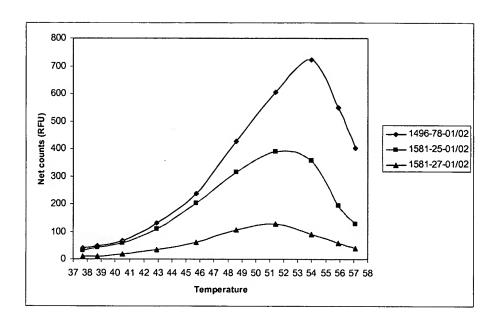
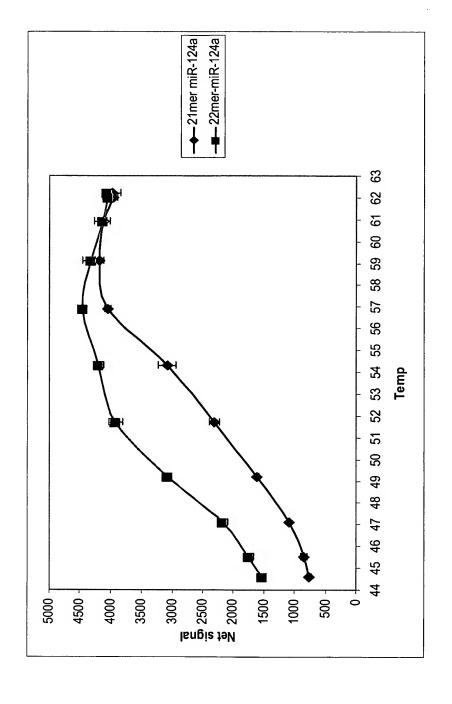
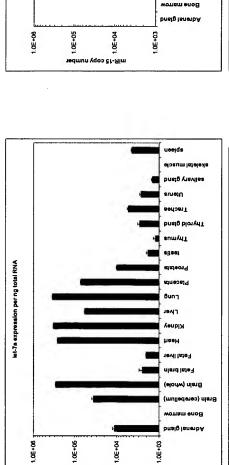


Figure 22





let-7a copy number

miR-15 expression per ng total RNA

elosum lateleda

selivery gland

Thyrold gland

miR-125b expression per ng total RNA

emetU

Trachea

Thymus

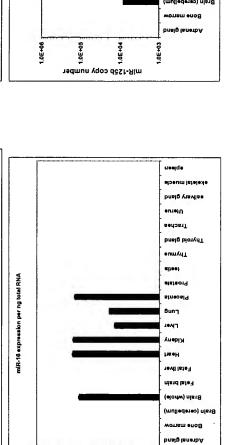
Bung

Liver Kideny

Heart

Fetal liver

Retal brain



ueejds

euretU

Traches

трутив

siteet

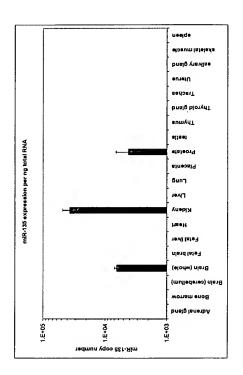
Kideny

Fetal liver

skeletal muecle

ealivary gland

Dasig bionydT



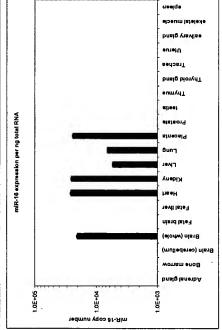


Fig. 23

## Fig. 24

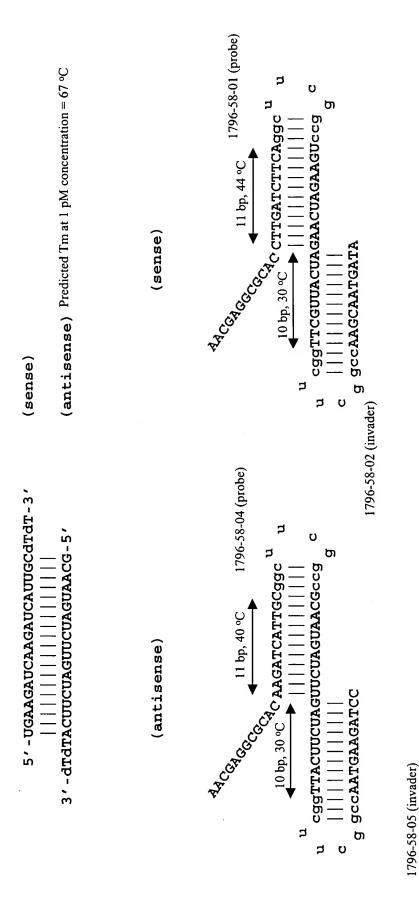
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tgtgccaactatacaact-3'	
CI.CC.	
CTACTACCT	CAcgacg t
<sub>t</sub> tgtgccAACTATACAACT-3'	
t       taacaa	gctgc <sup>t t</sup>
t	AGII

* CCGTCGCTGCGT CTACT t gtgccAACTATACAACT-3,	
CTACT	ACCTCAcgacg <sub>t</sub>
t gtgccAACTATACAACT-3,	
t	Tgctgc t °

5'-CCGTCGCC	
t gtgccAACTATACAAC  t            t cacg-	CTACTACCTCAcgacgt CT-3'      -ctgc t

UUGAUAUGUUGGAUGAUGAGU

# Figure 25



27/27

1796-58-03 (arrestor)

ugaagancaaggugcgc

1796-58-06 (arrestor)

gcaaugaucuugugcgc